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sub
E2 } D3 34. (Amended) The method of claim 32 wherein said one or more growth factors in the culture medium prepared in (b) is a fibroblast growth factor.

sub
E3 } D4 37. (Amended) The method of claim 26 wherein prior to (d) at least one subsequent cell culture is prepared by combining said neural stem cell progeny with fresh culture medium containing [at least] one or more growth factors capable of inducing multipotent neural stem cell proliferation to proliferate said daughter multipotent neural stem cells to produce more progeny which include more daughter multipotent neural stem cells.

Add
E5 } ✓
Please cancel Claim 38 without prejudice or disclaimer.

REMARKS

Amendments to the Claims:

The claims are amended to make them more concise.

Review of Application for Errors

Applicants acknowledge the Examiner's request that the application be reviewed for errors. Such a review was previously undertaken and errors in the application corrected as indicated in the amendment filed May 23, 1997.

Corrected Oath/Declaration

A corrected oath/declaration, which refers to the priority applications, is being executed by the inventors and is forthcoming.

Rejections Under 35 U.S.C. § 112

The Examiner maintained the rejection of claim 27 under § 112, 1st paragraph as not being enabled by the specification because the "longevity of

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expression" has not been demonstrated, and it has not been shown whether "sufficient levels can be expressed for the requisite length of time to achieve any therapeutic effect".

Claim 27 is directed to a method of transplanting neural stem cell progeny to a host which comprises obtaining a multipotent neural stem cell from mammalian neural tissue, proliferating the neural stem cell in a culture medium containing a growth factor that induces multipotent neural stem cell proliferation, genetically modifying the neural stem cell progeny to express a biological agent selected from the group consisting of growth factors, growth factor receptors, neurotransmitters, neurotransmitter synthesizing genes, neuropeptides, and chromaffin granule amine transporter, and transplanting the multipotent neural stem cell progeny into the host.

The specification provides ample enablement for this claim. Methods of obtaining multipotent neural stem cells from host tissue are disclosed on page 20, line 21 to page 21, line 16, and detailed in Examples 1 and 2. Methods of proliferating multipotent neural stem cells in a growth factor containing culture medium are disclosed on page 24, line 23 to page 28, line 22, and detailed in Examples 3 to 6. Methods of genetically modifying multipotent neural stem cell progeny are disclosed on page 32, line 22 to page 35, line 26, and detailed in Examples 18, 19, 21 to 23. Methods of transplanting multipotent neural stem cell progeny are disclosed on page 36, line 10 to page 42, line 13, and detailed in Examples 14 and 15. Accordingly, the requirements of 35 U.S.C. § 112, 1st ¶ have been satisfied.

With regards to the particular levels of expression the biological agent and the longevity of expression, Applicants point out that Claim 27 does not recite these particular features. Therefore, it is believed that the rejection of Claim 27 is improper. In any event, the issue of level and longevity of transgene expression was already addressed in Applicants' previous response where it was pointed that the Friedman paper, relied upon by the Examiner to support the § 112 rejection,

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states that "precursor CNS cells or 'stem' cells, can be genetically modified and grafted to an injured or diseased CNS to replace an aberrant, injured or degenerating neural function", and that such genetically modified cells can "give rise to developmentally appropriate structures after implantation" and express a transgene "for very long periods in the fully developed CNS." (Friedmann, p. 212, col. 2). Therefore, it is believed that the Examiner's rejection has already been adequately addressed. The Examiner did not state why Applicants' previous discussion of the Friedmann paper was not sufficient to overcome the § 112 rejection of claim 27. If, in view of the present Amendment, the Examiner still maintains this rejection, Applicants respectfully request the Examiner provide reasons why the rejection is maintained.

The Examiner rejected Claim 38 under § 112, 1st ¶, on the basis that the specification does not support the cell numbers claimed and that the specification fails to disclose or contemplate methods for measuring the numbers of multipotent stem cells obtained at any particular passage. Applicants maintain that there is more than ample disclosure in the specification on how to achieve a non-primary cell culture containing at least a 10-fold increase in numbers of multipotent neural stem cells over the primary culture. The specification teaches that these cells can be continuously passaged until the desired number of cells is obtained. Additionally, the approximate number of multipotent neural stem cells generated at each passaged can be determined by the number of neurospheres that form in response to growth factors that induce multipotent neural stem cell proliferation. However, in the interest of furthering prosecution, Claim 38 has been canceled without prejudice or disclaimer.

For the above reasons, it is believed that the rejections under § 112, 1st ¶, should be withdrawn.

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Rejections Under 35 U.S.C. § 102

Claim 26

The Examiner maintained the rejection of Claim 26 under § 102(a) as being anticipated by Lubetzki. For a proper rejection under § 102(a), every claim feature must be disclosed in a single prior art reference. Step (b) of claim 26 recites that a culture medium is prepared that contains "one or more growth factors capable of inducing multipotent neural stem cell proliferation". Step (c) recites that a multipotent neural stem cell is induced to proliferate.

With regards to step (b) of claim 26, it is the Examiner's position that the PDGF-containing culture medium of Lubetzki discloses all of the features of this step. However, merely preparing a growth-factor containing medium does not satisfy all the features of step (b). The growth factor, or factors, must be capable of inducing multipotent neural stem cell proliferation. Different growth factors have different effects on different cells. While PDGF is known to induce proliferation of 0-2A progenitor cells, which are capable of giving rise to astrocytes and oligodendrocytes (but not neurons), this growth factor has not been reported to induce proliferation of multipotent neural stem cells, which are capable of giving rise to astrocytes, oligodendrocytes, and neurons. In fact, Applicants have shown that PDGF actually inhibits multipotent neural stem cell proliferation. Page 92, lines 22 to 26, of Applicants' disclosure, discusses the effects of various "regulatory factors" on growth-factor induced proliferation of multipotent neural stem cells, and states that PDGF "significantly reduced the numbers of neurospheres generated in all of the proliferative factors or combinations of proliferative factors tested." The results are shown in Table II on page 93 of the specification. Because the Lubetzki reference does not teach a culture medium containing a growth factor that induces multipotent neural stem cell proliferation, it does not teach the features of step (b).

Regarding step (c) of Claim 26, which recites that a multipotent neural stem cell is induced to proliferate, the Examiner stated that "since the cell

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population was derived from brains, the transplanted cell population presumably contained some stem cells". However, step (c) of Claim 26 does not merely call for the presence of stem cells in the cell culture, it calls for the proliferation of multipotent neural stem cells. The possibility that Lubetzki's cultures may have contained a few quiescent stem cells, does not teach or suggest the features of step (c). The Examiner has not asserted that the methods of Lubetzki resulted in proliferation of multipotent neural stem cells, only that "progenitor cells were induced to proliferate... since PDGF was added". As previously mentioned, PDGF induces O-2A progenitor cells to proliferate, whereas it inhibits multipotent neural stem cell proliferation. Thus, the Examiner has not shown how the features of step (c) of Claim 26 are disclosed in the Lubetzki reference.

For these reasons, a rejection under § 102(a) based on the Lubetzki reference can not be maintained.

Rejections under 35 U.S.C. § 103

Claim 27

The Examiner maintained the rejection of Claim 27 under 35 U.S.C. § 103 as being unpatentable over Lubetzki and Gage. The Examiner did not indicate why the Applicants' argument presented in the previous response that PDGF has not been shown to induce proliferation of multipotent neural stem cells was not persuasive. The Examiner merely stated that she disagreed with Applicants' statement that a "neuron" is a "nerve cell", and maintained that "a neuron is a type of nerve cell". Attached as Appendix A is a page from the glossary of "Human Physiology", 5th Edition, by Vander, Sherman and Luciano, published by McGraw Hill (1990). It will be noted that under the definition of "Neuron" it states: "see nerve cell". Under "nerve cell" it states that such cells are specialized to conduct electrical signals (something that is not true of glia and oligodendrocytes).

In all events, Applicants maintain that Lubetzki does not teach culture conditions that induce multipotent neural stem cell proliferation (features recited in

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Claim 26, from which Claim 27 depends), and that Gage does nothing to cure this deficiency.

The initial burden is on the Examiner to establish a *prima facie* case of obviousness which, as set forth in M.P.E.P. § 706.02(j), requires:

...the examiner to provide some suggestion of the desirability of doing what the inventor has done. "To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985).

The Examiner has not stated how the combination of Lubetzki with Gage would have suggested the desirability of using growth factors to induce multipotent neural stem cells to proliferate *in vitro*. Accordingly, a *prima facie* case of obviousness has not been established.

The Examiner rejected Claims 32 to 51 under 35 U.S.C. § 103(a) as being unpatentable over Lubetzki as applied to Claim 26, and further in view of Olson and Pezzoli. It is assumed that the Examiner also intended to include the Gage reference in this rejection, as Gage is relied upon in the rejection of claims 41 to 47. Applicants respond accordingly.

Claims 32 and 34

The Examiner acknowledges that the Lubetzki reference does not teach using any of the growth factors listed in Claims 32 and 34, and relies on the Olson reference for disclosing that some of these growth factors "can stimulate growth of fetal brain tissue."

There is no reason why one of ordinary skill in the art would combine the teachings of Lubetzki with Olson. Lubetzki is concerned solely with proliferating O-2A progenitor cells and transfecting these cells with the LacZ gene so that their

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ability to myelinate upon transplantation can be observed. The Olson reference, on the other hand, is concerned with the *in vivo* administration of growth factors to enhance neuron graft survival. The combined teachings provide no motivation to substitute the PDGF used by Lubetzki with the growth factors discussed in the Olson reference, because there is no reason to believe that a growth factor that promotes neuron survival would also induce O-2A cell proliferation. A particular cell type may have a completely different response to a particular growth factor than a different cell type. Moreover, none of the cited references concerns growth factor induced proliferation of multipotent neural stem cells *in vitro*, nor do they suggest that this would be possible.

Claim 33

The Examiner relies on the Pezzoli reference for disclosing that "EGF may have a mitogenic activity *in vivo*". Movement Disorders, is a quarterly journal. The Pezzoli reference was published in the fourth quarter volume of that journal in 1991. This application was previously amended, in compliance with 35 U.S.C. § 120, to contain a specific reference to an application filed in July 8, 1991 (see amendment filed December 6, 1995). The priority application was published before the Pezzoli reference and discloses EGF-induced proliferation of multipotent neural stem cells, and transplantation of the proliferated cells. Therefore, it is believed that the Pezzoli reference is not prior art.

Even though Pezzoli is believed not to be prior art, for the sake of completeness, Applicants discuss how this reference differs from the claimed invention. The Pezzoli reference discloses that the damage caused by mechanical or chemical lesions in the dopaminergic pathways between the mesencephalon and the striatum can be significantly reduced by *in vivo* administration with EGF. While the reference discloses that it is a possibility that EGF may have a mitogenic effect *in vivo*, it states that the "most plausible" explanation for the effects EGF had could be explained by a "neurotrophic effect

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on... dopaminergic neurons..." (see p. 285, col. 2). This teaching would not have provided one of ordinary skill in the art, at the time of applicants' invention, with a reasonable expectation of success that EGF would have a proliferative effect in vitro on multipotent neural stem cells. Therefore, there would have been no motivation to combine the Pezzoli and Lubetzki references in the manner suggested by the Examiner.

Claim 35

Claim 35 is directed to using the specific combination of EGF and FGF to induce proliferation of multipotent neural stem cells *in vitro*. The specification teaches that this combination significantly increases the rate of stem cell proliferation (see sentence bridging pages 51 and 52 of specification). The cited prior art does not teach or suggest this combination.

Claim 36

The Examiner states that "'substantially serum free' is the standard culture condition, lacking evidence to the contrary." This is not a proper rejection under § 103. The initial burden is on the Examiner to establish a *prima facie* case of obviousness which, as set forth in M.P.E.P. § 706.02(j) (and reproduced above). The Examiner's mere assertion that "substantially serum free" is a standard culture condition does not rise to the level of a *prima facie* showing of obviousness as enunciated in the M.P.E.P. While, it is true that in some situations, an Examiner can rely on "common knowledge" in the art or "well known" prior art, without citing a specific reference that teaches the allegedly obvious feature of the claimed invention (see M.P.E.P. § 2144.03), this is not one of those situations. While "substantially serum free" culture conditions are known in the prior art, they are not necessarily "standard". For Example, the cultures of Lubetzki, cited by the Examiner, contained either 1% or 10% fetal calf serum (see p. 87 under the heading "Cell Cultures"). At the time of the invention, serum was

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commonly used in neural cell cultures, because it provided nutrients that helped certain neural cell types to survive culture conditions.

Claim 37

Regarding claim 37, the Examiner stated that "repetitive rounds of cell culture of primary cell cultures is routinely done by those of ordinary skill in the art". Again, the Examiner has not based this rejection on the teaching of any reference. It is admitted that many different cell types, particularly cell lines that have been immortalized with oncogenes, can be passaged to produce non-primary cultures. However, Applicants are the first to demonstrate that a multipotent neural stem cell, capable of giving rise to astrocytes, neurons and oligodendrocytes, can be induced to proliferate *in vitro*, in the presence of one or more growth factors, to produce daughter multipotent neural stem cells which can be passaged into fresh growth factor containing medium and induced to further proliferate.

Claim 48

This claim is directed to the use of a host's own tissue to obtain the multipotent neural stem cell progeny which are then transplanted back into the host (i.e. autologous transplantation). The Lubetzki reference concerns proliferation and possible transplantation of O-2A progenitor cells. The reference does not suggest the possibility of using autologous cells. In any event, as previously discussed, O-2A cells are not the same as multipotent neural stem cells. While the Gage patent teaches the use of autologous cells wherever feasible (col. 14, lines 39 to 42), it fails to teach or suggest using the progeny of multipotent neural stem cells derived from a patient's own tissue for transplantation purposes. Gage clearly indicates that (at the time the Gage application was filed) there was a "paucity of replicating non-transformed cell culture systems" which could be used for neural transplantation purposes (see

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col. 14, lines 61-66). Thus, the Gage method uses fibroblasts (non-neural cells) for transplantation. The combination of Gage and Lubetzki does not teach nor suggest the invention as recited in Claim 48.

Claim 49

Claim 49 is directed to the use of juvenile or adult mammalian neural tissue. The Examiner apparently relies on the Lubetzki reference for teaching this feature, stating that "the type of neural tissue transfected is obvious over the transfection of mature and day old cells of Lubetzki". The ability to proliferate O-2A progenitor cells obtained from adult tissue (as taught by Lubetzki) would not have led one of ordinary skill in the art, at the time of Applicants invention, to believe that multipotent neural stem cells capable of giving rise to astrocytes, oligodendrocytes, and neurons, could be obtained from adult tissue and proliferated *in vitro*. At the time of Applicants invention, it was commonly believed that juvenile and adult mammalian CNS did not contain multipotent neural stem cells (see p. 3, lines 13-16 of specification).

For the above reasons, the rejection of claims 32 and 51 in view of Lubetzki, Olson, Pezzoli, and Gage should be withdrawn. Additionally, it should be pointed out that claims 32 through 51 all depend (directly or indirectly) from claim 26, which requires growth factor induced proliferation of multipotent neural stem cells *in vitro*. The Examiner relied on Lubetzki for teaching this feature. However, for reasons already presented, this reliance is misplaced.


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CONCLUSION

For the foregoing, it is believed that the claims of this application are patentable. Favorable reconsideration is respectfully requested.

Respectfully submitted,

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APPENDIX A

FIFTH
EDITION

HUMAN PHYSIOLOGY

THE MECHANISMS OF BODY FUNCTION

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- and multineuronal pathways: *see also* primary motor cortex
- motor end plate** specialized region of muscle-cell plasma membrane that lies directly under axon terminal of a motor neuron
- motor neuron efferent neuron** that innervates skeletal muscle
- motor neuron pool** all the motor neurons for a given muscle
- motor potential** electrical activity that can be recorded over motor cortex about 50 to 60 ms before a movement begins
- motor system** those CNS parts that contribute to control of skeletal-muscle movements
- motor unit** motor neuron plus the muscle fibers it innervates
- mRNA** *see* messenger RNA
- mucin (MU-sin)** protein that, when mixed with water, forms mucus
- mucosa (mü-KO-sah)** three layers of gastrointestinal tract wall nearest lumen, that is, epithelium, lamina propria, and muscularis mucosa
- mucus** highly viscous solution secreted by mucous membranes
- Müllerian duct (mul-AIR-ee-an)** part of embryo that, in a female, develops into reproductive system ducts, but in a male, degenerates
- Müllerian inhibiting hormone (MIH)** protein secreted by fetal testes that causes Müllerian ducts to degenerate
- multineuronal pathways** pathways made up of chains of neurons functionally connected by synapses; specifically, descending motor pathways that synapse in basal ganglia and other subcortical nuclei and in the brainstem; only final neuron of chain reaches region of motor neurons; extrapyramidal system; also called *multisynaptic pathways*
- multiunit smooth muscle** smooth muscle that exhibits little, if any, propagation of electrical activity from fiber to fiber and whose contractile activity is closely coupled to its neural input
- muscarinic receptor (mus-cur-IN-ik)** acetylcholine receptor that responds to the mushroom poison muscarine; located on smooth muscle, cardiac muscle, some CNS neurons, and glands
- muscle** number of muscle fibers bound together by connective tissue
- muscle fatigue** decrease in mechanical response of muscle with prolonged stimulation; *compare* psychological fatigue
- muscle fiber** muscle cell
- muscle-spindle stretch receptor** capsule-enclosed arrangement of afferent nerve fiber endings in skeletal muscle; sensitive to stretch
- muscle tension** force exerted by a contracting muscle on an object
- muscle tone** degree of resistance of muscle to passive stretch
- mutation (mu-TAY-shun)** any change in base sequence of DNA that changes genetic information
- mV** *see* millivolt
- myasthenia gravis (my-as-THEE-nee-ah GRAH-vis)** autoimmune neuromuscular disease associated with skeletal-muscle weakness and fatigue; due to destruction of skeletal-muscle receptors for acetylcholine
- myelin (MY-uh-lin)** insulating material covering axons of many neurons; consists of layers of myelin-forming cell plasma membrane wrapped around axon
- myenteric plexus (my-en-TER-ik PLEX-us)** nerve cell network between circular and longitudinal muscle layers in esophagus, stomach, and intestinal walls
- myo- (MY-oh)** pertaining to muscle
- myoblast (MY-oh-blast)** embryological cell that gives rise to muscle fibers
- myocardial infarction** *see* heart attack
- myocardium (my-oh-CARD-ee-um)** cardiac muscle, which forms heart walls
- myoepithelial cell (my-oh-ep-ih-THEE-lee-al)** specialized contractile cell around certain exocrine glands; contraction forces gland's secretion through ducts
- myofibril (my-oh-FI-bril)** thick or thin contractile filament in cytoplasm of striated muscle; myofibril clusters are arranged in repeating sarcomere pattern along longitudinal axis of muscle
- myogenic (my-oh-JEN-ik)** originating in muscle
- myoglobin (my-oh-GLOW-bin)** muscle-fiber protein that binds oxygen
- myometrium (my-oh-ME-tree-um)** uterine smooth muscle
- myosin (MY-oh-sin)** contractile protein that forms thick filaments in muscle fibers
- myosin ATPase** enzymatic site on globular head of myosin that catalyzes ATP breakdown to ADP and P_i , releasing the chemical energy used to produce force of muscle contraction
- myosin light-chain kinase** smooth-muscle protein kinase; when activated by Calmodulin, phosphorylates myosin
- NaCl** sodium chloride
- Na^+ , K^+ -ATPase pump** primary active-transport carrier protein that splits ATP and releases energy that is used to transport sodium out of cell and potassium in
- natriuretic hormone (nat-rye-yur-ET-ik)** hormone that is secreted by atrium of heart; inhibits sodium reabsorption in renal tubule
- natural antibody** antibody to erythrocyte antigens A or B; are present without prior exposure to antigen
- natural killer (NK) cell** type of lymphocyte that binds relatively nonspecifically to cells bearing foreign antigens and kills them directly; no MHC restriction
- nearsighted** vision defect because eyeball is too long for lens, so that images of distant objects are focused in front of retina
- negative balance** substance loss from body exceeds gain and total amount in body decreases; also used for physical parameters such as body temperature and energy; *compare* positive balance
- negative feedback** aspect of control systems in which system's response opposes input to system; *compare* positive feedback
- nephron (NEF-ron)** functional unit of kidney; has vascular and tubular component
- nerve** group of many nerve fibers traveling together in peripheral nervous system
- nerve cell** cell specialized to initiate, integrate, and conduct electric signals; also called neuron
- nerve fiber** *see* axon
- nerve growth factor** peptide that stimulates growth and differentiation of some neurons
- net** amount remaining after opposing quantities are subtracted from each other; final amount
- neuroeffector junction** "synapse" between a neuron and muscle or gland cell
- neuroglia** *see* glial cell
- neurohormone** chemical messenger that is released by a neuron and travels in bloodstream to its target cell
- neuromodulator** chemical messenger that acts on neurons, usually by a second-messenger system, to alter response to a neurotransmitter
- neuron (NUR-on)** *see* nerve cell
- neuropeptide** family of at least 50 neurotransmitters composed of two or more amino acids; often functions as chemical messengers in nonneural tissues
- neurotransmitter** chemical messenger used by neurons to communicate with each other or with effectors
- neutrophil (NEW-tro-fil)** polymorphonuclear granulocytic leukocyte whose granules show preference for neither eosin nor basic dyes; functions as phagocyte and releases chemicals involved in inflammation
- neutrophil exudation (ex-ooH-DAY-shun)** amoeba-like movement of neutrophils from capillary lumen to tissue extracellular space
- NH_3** ammonia
- NH_4^+** ammonium ion
- nicotinic receptor (nik-oh-TIN-ik)** acetylcholine receptor that responds to nicotine; primarily, receptors at motor end plate and on postganglionic autonomic neurons
- nociceptor (NO-sih-sep-tor)** sensory receptor whose stimulation causes pain
- node of Ranvier (RAHN-vee-ii)** space between adjacent myelin-forming cells along myelinated axon where axonal plasma membrane is exposed to extracellular fluid
- nonpolar molecule** molecule containing pre-